Oxidation of Mn(II) catalyzed by spore coats of a marine *Bacillus*, strain SG-1

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BACKGROUND

Manganese oxidation catalyzed by bacteria may lead to a different sequence of oxidation states, minerals, and rates when compared to non-biological manganese oxidation under the same conditions of temperature, Mn(II) concentration, and time. Manganese oxide and hydroxide minerals are very reactive and play an important role in the mobility and bioavailability of heavy metals and organic contaminants. Therefore, an understanding of the products and rates of manganese oxidation, by both biological and non-biological processes, is essential to interpret biogeochemical cycles, as well as to recognize biosignatures in rocks.

Manganese oxidation may proceed directly to Mn(IV) in a one-step, 2-electron transfer, or from Mn(II) to Mn(IV) in a 1-electron transfer, with Mn(III) intermediates. Laboratory experiments of abiotic manganese oxidation provide evidence for the first step of a 2-step oxidation, leading to the formation of Mn_3O_4 or MnOOH:[1,2]

$$3\ Mn^{+2} + 3\ H_2O + 1/2\ O_2 = Mn_3O_4 + 6\ H^+ \qquad \text{EQ 1}$$

$$Mn_3O_4 + 2H^+ = 2MnOOH + Mn^{+2} \qquad \text{EQ 2}$$

The Mn_3O_4 should theoretically disproportionate to MnO_2 over time:

$$Mn_3O_4 + 4H^+ = MnO_2 + 2Mn^{+2} + 2H_2O \qquad \text{EQ 3}$$

but the disproportionation reaction mechanism has not yet been entirely demonstrated in the laboratory.

Laboratory experiments of bacterially catalyzed manganese oxidation by the marine *Bacillus* spore SG1, isolated from a manganese coating on a sandgrain from marine sediments[3], have shown evidence for both 2-step and 1-step manganese oxidation. Hastings and Emerson observed the formation of Hausmannite (Mn_3O_4) which aged to MnO_2 during a time scale of weeks.[4] Mandernack et al. found evidence for a 1-step , 2 electron oxidation.[5] Their evidence was based on oxygen isotopic analysis, x-ray diffraction and oxidation state analysis.

There is also strong evidence that mineral oxide surfaces not only promote the sporulation of SG-1 spores[6] but can also catalyze the oxidation of dissolved Mn(II) in a pure chemical reaction.[7,8] It is therefore to be expected that a rather complicated interplay of biologically mediated and pure chemical reactions may lead to a very complex sequence of pathways and products being formed. For most of the biological and chemical experiments so far, analytical differentiation between involved chemical species has been carried out by indirect methods like titrations or operational

reduction of the oxides formed. Thus, all of these methods can give only an average oxidation state of the minerals and species present.

Our objective is to achieve a more complete understanding of reaction steps involved during oxidation of Mn(II) by oxygen, catalyzed by proteins in the spore coats. Because of its rich spectral signature, XANES (X-ray absorption near edge structure) at the Mn L-edge is particularily well suited to address this question. We were using TEY-XANES (total electron yield) and STXM (Scanning Transmission X-ray Microscope) on beam line 7.0.1 and XM-1 (X-ray microscope 1) on beam line 6.1.2 to analyze the spatial distribution of Mn charge states on spores of *Bacillus* SG-1 directly.

We chose to repeat one of the experiments of Mandernack et al in which one of the early oxidation products was the mineral birnessite, [(Na,Ca)Mn_7 O_{14} x $2H_2O$]. We synthesized a non-biological sample of birnessite to compare with the biological experiments.

METHODS

The birnessite mineral was synthesized by the method of McKenzie.[9] The bacterial spores were grown and cleaned according to Rosson and Nealson.[10] A solution of $MnCl_2$ (10mM) with 75% seawater and a HEPES buffer at pH 7.5 were inoculated with a thick suspension of spores (~ 10^{10} spores/mL). In the first experiment, the spores were allowed to react for 3 days and 6 days, and the suspensions were sampled directly at the STXM beamline. In the second experiment, samples were reacted for 1 day, 2 days and 5 days, washed, and frozen. These frozen samples were then thawed and sampled at the STXM beamline. Suspensions from all of the reactions showed a change in spore color from light to dark. Controls with no spores showed no evidence of manganese oxidation. Two μl of these suspensions were sandwiched between two Si_3N_4 wafers which were then fixed to aluminum sample holders. Details about sample preparation and the microscopic techniques used are given in Rothe et al.[11]

RESULTS

Figure 1 shows a soft x-ray image of a wet spore sample, taken at the XM-1 (BL 6.1.2). The spore structure shows a dark interior, surrounded by a lighter region. Some dark irregular protrusions can be seen along the exterior of the light region. These protrusions were assumed to be Mn-precipitates. However, taking images at photon energies selective for Mn, we were unable to identify these structures as Mn-precipitates using XM-1.

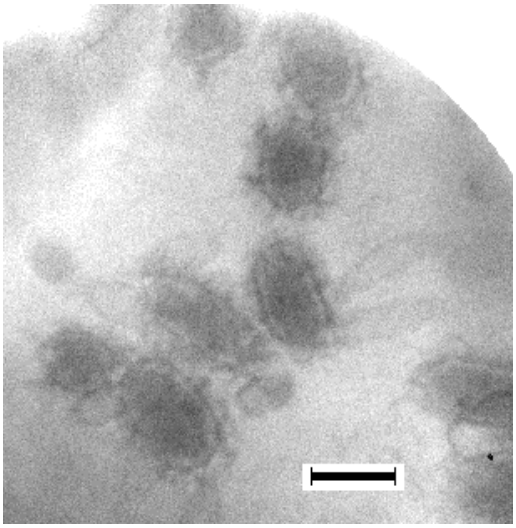

Figure 1. XM-1 image of SG-1 spores. Bar is 1 μm.

Complementary to XM-1, stacks of images at different photon energies were taken with STXM to get better spectral information of expected Mn-coatings around spore cells. Figure 2 shows the results obtained from a 5 days old spore preparation. Surprisingly, Mn-precipitates found on different spore cells from one sample were not uniform but seemed to vary from spore to spore. A similar picture was found for a 2 days old preparation indicating that reaction kinetics are complex. Comparing the STXM XANES spectra to TEY-XANES spectra of

reference compounds (Fig. 3), we have found spectra which almost perfectly coincide with Mn(II) (spectrum #1 in Fig. 2) and Mn(IV) (spectrum #4 in Fig.2). Although spectrum 1 (Fig. 2) seems to coincide pretty well with the TEY-XANES spectrum of MnF_3 (Fig.3) we are not yet sure whether we are seeing a pure Mn(III) valence state. Theoretical calculations using a modified version of deGroot's atomic multiplet theory[12] have shown that the spectrum of Mn_2O_3 (Fig.3) probably represents Mn(III) best. MnF_3 is a very reactive compound and may have been oxidized already to some degree at the surface of single grains. We are currently verifying a true Mn(III) reference sample by collecting spectra of high purity Mn_2O_3 and freshly synthesized manganite (γ-MnOOH).

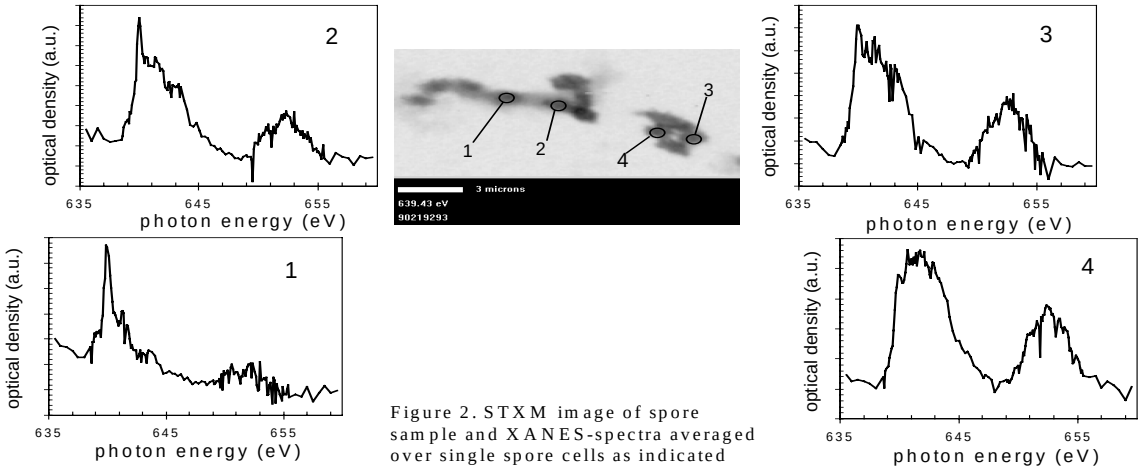

Figure 2. STXM image of spore sample and XANES-spectra averaged over single spore cells as indicated

CONCLUSIONS

We have shown that STXM can be used to determine the average oxidation state of Mn precipitates on individual spore cells of size ~1 μm. Spore structure was verified using XM-1. Oxidation was rapid, with Mn(IV) present on some spores within 2 days. This supports the argument for biological oxidation. Reasons for observed variations in oxidation states of attached Mn between single spore cells are manifold. Accessibility of outer spore cell membranes within the suspensions for Mn(II) and oxygen as well as spore dependent differences in membrane structure itself may account for the found heterogeneity. Because of uncertainty in our standards, we cannot decide yet that we were seeing a pure Mn(III) valence state. Spores or specific sites on spore coats (proteins) are probably initializing a sequence of adsorption and/or heterogeneous nucleation of Mn(II). The possibility of a stable Mn(III) intermediate implies that either chemical oxidation occurs simultaneously with the biological oxidation, that the biological oxidation occurs in 2 steps instead of one, or that some other process is reducing the Mn(IV) to Mn(III). Future experiments, with better controls, standards, and measurement of chemical changes with time, should help clarify these issues.

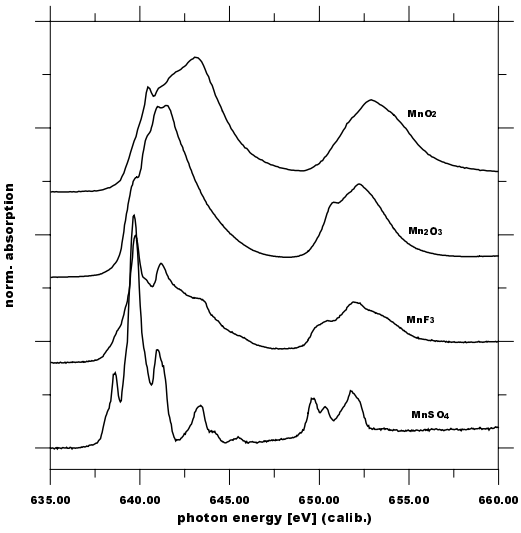

Figure 3. Mn $L_{2,3}$ TEY-XANES of reference compounds

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